Host-microbe interactions that shape the pathogenesis of *Acinetobacter baumannii* infection

Brittany L. Mortensen and **Eric P. Skaar**[*]
Department of Pathology, Microbiology and Immunology, Vanderbilt University School of Medicine, Nashville, TN 37232

Summary

Acinetobacter baumannii is an opportunistic pathogen that has emerged as a prevalent source of nosocomial infections, most frequently causing ventilator-associated pneumonia. The emergence of pan-drug resistant strains magnifies the problem by reducing viable treatment options and effectively increasing the mortality rate associated with *Acinetobacter* infections. In light of this rising threat, research on *A. baumannii* epidemiology, antibiotic resistance, and pathogenesis is accelerating. The recent development of both *in vitro* and *in vivo* models has enabled studies probing the host-*Acinetobacter* interface. Bacterial genetic screens and comparative genomic studies have led to the identification of several *A. baumannii* virulence factors. Additionally, investigations into host defense mechanisms using animal models or cell culture have provided insight into the innate immune response to infection. This review highlights some of the key attributes of *A. baumannii* virulence with an emphasis on bacterial interactions with the innate immune system.

Introduction

Acinetobacter baumannii is a Gram-negative, aerobic coccobacillus that belongs to a genus comprised of both environmental species and opportunistic pathogens. Interestingly, *A. baumannii* is an opportunistic pathogen that unlike others in the genus is not ubiquitous in nature and exhibits a low human carrier rate. This resilient organism is notorious for its ability to withstand desiccation and disinfection and to persist in the hospital environment. Contributing to the fortitude of *A. baumannii* is its propensity to form biofilms on a number of abiotic surfaces, including catheters, ventilators, and other medical devices, enhancing bacterial transmission (Vidal *et al.*, 1996; Tomaras *et al.*, 2003; Pour *et al.*, 2011). This organism is a particular challenge in intensive care units where it is responsible for a diverse range of infections including ventilator-associated pneumonia, skin and wound infections, urinary tract infections, and bacteremia (Doyle *et al.*, 2011). In addition to ventilator-associated pneumonia, there have also been reports of severe community-acquired pneumonia caused by *A. baumannii* (Falagas *et al.*, 2007). The escalating number of infections involving multi-drug and pan-drug resistant strains necessitates the development of new treatment options against this emerging threat. Considerable progress has been made towards understanding the epidemiology, mechanisms of antibiotic resistance, and persistence of *A. baumannii* in the hospital environment, and there are several recent reviews on these topics (Peleg *et al.*, 2008a; Garnacho-Montero *et al.*, 2010; Gordon *et al.*, 2010; Durante-Mangoni *et al.*, 2011). In contrast, much less is known regarding the pathogenesis of *A. baumannii* disease. In fact, we are only beginning to uncover the immune

Corresponding author information: Eric P. Skaar, PhD, MPH Department of Pathology, Microbiology and Immunology Vanderbilt University School of Medicine A5102 MCN, 1161 21st Avenue South Nashville, TN 37232-2363 Tel: 615-343-0002 Fax: 615-343-7392 eric.skaar@vanderbilt.edu.

pathways that are critical to host defense towards *A. baumannii,* and a small number of virulence factors have been identified in this organism. This review summarizes the current understanding of the pathogenesis of *A. baumannii* infections with an emphasis on the innate immune response to this pathogen.

A. baumannii elicits a pro-inflammatory immune response

Due to vigilant monitoring by immune cells, penetration of *A. baumannii* into the vertebrate host does not go undetected, and recognition by sentinel receptors triggers a rapid immune response. Bacterial pathogen-associated molecular patterns (PAMPs) are detected by innate immune receptors on host cells. As a result, intracellular signaling pathways promote transcription, processing, and secretion of inflammatory mediators. Although there are multiple inflammatory signaling cascades leading to cytokine and chemokine secretion, *A. baumannii* PAMPs are known to activate the NF- B and MAPK pathways (March *et al.*, 2010). This induces release of numerous chemokines, including macrophage inflammatory protein 2 (MIP-2), monocyte chemoattractant protein (MCP-1), and keratinocyte-derived chemokine (KC)/IL-8, and pro-inflammatory cytokines such as TNF- , IL-1 , and IL-6 (Knapp *et al.*, 2006; van Faassen *et al.*, 2007). Bearing in mind the complexity that comprises the immune response to a given pathogen, the range of pro-inflammatory mediators and pathways activated during *A. baumannii* infection certainly extends beyond those listed here.

Like many Gram-negative bacteria, the LPS of *A. baumannii* is highly immunostimulatory (Garcia *et al.*, 1999). TLR4 and its co-receptor CD14 recognize *A. baumannii* LPS, leading to NF- B activation, secretion of MIP-2 and KC/IL-8, and subsequent neutrophil recruitment (Knapp *et al.*, 2006; Erridge *et al.*, 2007). Of the three components of LPS (lipid A, core polysaccharide, and O-antigen), lipid A is the main immune-activating portion of the molecule. Remarkably, several colistin-resistant mutants of *A. baumannii* have been described with mutations in lipid A biosynthetic genes that eliminate LPS expression and increase membrane permeability (Moffatt *et al.*, 2010). The ability of *A. baumannii* to permit loss of LPS without a concomitant loss of viability is a surprising feature of this organism. Finally, the loss of the glycosyltransferase *lpsB* truncates LPS at the core polysaccharide and reveals that full-length LPS is necessary for serum resistance and survival *in vivo*, though immunostimulatory properties of this truncated LPS have not been explored (Luke *et al.*, 2010).

The inflammatory response to *A. baumannii* does not appear to be exclusively dependent on TLR4. TLR2 is also involved, although its relative contribution is less understood. TLR2-deficient mice exhibit accelerated neutrophil influx, improved clearance of *A. baumannii,* and reduced pro-inflammatory responses (Knapp *et al.*, 2006). This suggests that TLR2 signaling is detrimental to the host in the context of *A. baumannii* infection and may participate in anti-inflammatory activity. Others have shown in similar models that TLR2 activation is pro-inflammatory, up-regulating NF- B and secretion of IL-8 in response to *A. baumannii* infection (Erridge *et al.*, 2007; March *et al.*, 2010). Clearly, further research is needed to elucidate the contribution of TLR2 to the pathogenesis of *A. baumannii* infection.

The armamentarium of recruited neutrophils controls A. baumannii infection.

A. baumannii-induced activation of the innate immune response stimulates chemokine secretion and subsequent recruitment of immune cells to the site of infection in both pneumonia and septicemia models. Neutrophils are the largest population of recruited cells and are required for controlling infection; however, macrophages and natural killer (NK)

cells have also been detected following *A. baumannii* challenge (van Faassen *et al.*, 2007; Breslow *et al.*, 2011; Tsuchiya *et al.*, 2011). NK cells participate in neutrophil recruitment through increased expression of KC/IL-8 (Tsuchiya *et al.*, 2011). Lymphocytes and granulocytes produce a number of antimicrobial factors in response to infection, including defensins, cathelicidins, reactive oxygen species (ROS), and reactive nitrogen species (RNS). ROS and the related myeloperoxidase as well as -defensin-2 have been assigned roles in *A. baumannii* killing, with a minor contribution by RNS (Knapp *et al.*, 2006; Qiu *et al.*, 2009; March *et al.*, 2010). Undoubtedly, there are additional immune cell effectors and antimicrobial peptides in the neutrophil arsenal that are relevant to *A. baumannii* infection.

Host-Acinetobacter interactions shape the composition of the immune response

The immune response is principally dependent on how the host interacts with a given pathogen. More specifically, the particular immune receptors that detect antigens or PAMPs are dictated by the host cell type and the subcellular location of these sentinel proteins. *A. baumannii* is a versatile pathogen that can adhere to and invade numerous cell types, yet different cell types display varying degrees of susceptibility to invasion (Choi *et al.*, 2008c). Diverse strains of *A. baumannii* also have distinct capacities for cell adherence and invasion (Lee *et al.*, 2006; de Breij *et al.*, 2010; Eijkelkamp *et al.*, 2011b). *A. baumannii* attaches to bronchial epithelial cells by means of short, fimbrial-like protrusions on the bacterial cell surface (Lee *et al.*, 2008). Following attachment, *A. baumannii* can invade epithelial cells in a microfilament- and microtubule-dependent, zipper-like mechanism (Choi *et al.*, 2008c). This interaction leads to host cell cytotoxicity. Specifically, during infection host epithelial cells up-regulate caspase-3, -8, -9, and poly[ADP-ribose] polymerase (PARP) that correlate with secretion of cytochrome c and apoptosis inducing factor (AIF) from the mitochondria (Choi *et al.*, 2005). The stimuli and signaling pathways implicated in cell death are not established; however, they involve imbalanced calcium homeostasis, pro-inflammatory cytokines, and oxidative stress (Smani *et al.*, 2011). Several significant questions regarding the lifecycle of *A. baumannii* remain to be answered. For instance, understanding the functional relevance of *A. baumannii* invasion and the intracellular trafficking patterns is crucial, as this can profoundly shape the host cell responses. In addition, the mechanisms whereby *A. baumannii* modulates virulence factor expression to adapt to the host environment are not clear.

The balance between anti- and pro-inflammatory responses affects pathogenesis

Although *A. baumannii* stimulates the pro-inflammatory immune response, it induces a weaker response than the less-pathogenic *A. junii* (de Breij *et al.*, 2010). This phenomenon is extended to individual strains and clinical isolates of *A. baumannii* that differ in virulence. More specifically, highly virulent strains induce more severe lung pathology and lower levels of anti-inflammatory cytokines without exhibiting increased bacterial organ burdens or dissemination (de Breij *et al.*, 2012). Therefore, there is a direct correlation between virulence of a given strain and the strength of the pro-inflammatory response. This observation also highlights the need to balance the pro- and anti-inflammatory responses to a pathogen such that there is sufficient inflammation to eradicate the invader while not severely injuring the host.

Relatedly, most patients with *A. baumannii* infection are immune-compromised, often in a heightened inflammatory state. Postsurgical and trauma patients have a pre-existent acute phase response characterized by the presence of proteins such as C-reactive protein, serum

amyloid A (SAA), and serum amyloid P and concurrent changes to the inflammatory response. In an acute phase response model, *A. baumannii* infection results in decreased pro-inflammatory cytokine secretion and decreased neutrophil recruitment to the lungs due at least in part to SAA (Renckens *et al.*, 2006). Additionally, in an allergic asthma model, *A. baumannii* infection suppresses allergic-like responses, most notably a decrease in eosinophil influx into the lung and reduction of Th2 cytokines (Qiu *et al.*, 2011). These studies indicate that the pre-existing immunological environment can impact the host response to *A. baumannii* and ultimately the outcome of infection.

Complement defenses in the serum are circumvented by *A. baumannii*

Complement is a major bactericidal component in serum that limits microbial dissemination. Typically, one of three pathways is activated leading to deposition of complement factors on the surface of bacteria and consequent bacterial lysis or opsonin-mediated phagocytosis. The alternative complement pathway is responsible for *A. baumannii* killing in human serum; however, clinically relevant strains can be resistant to complement activity. There is considerable debate over the mechanism behind the serum resistance of *A. baumannii*. Proposed models include bacterial-mediated inactivation of the alternative complement pathway inhibitor Factor H (Kim *et al.*, 2009), *A. baumannii* release of LPS (Garcia *et al.*, 2000), and the modification of peptidoglycan by the penicillin-binding protein PBP-7/8 (Russo *et al.*, 2009). Furthermore, the presence of surface polysaccharides or capsule protects Gram-negative bacteria from host antimicrobials in serum, and several strains of *A. baumannii* produce a polysaccharide capsule (Russo *et al.*, 2010; Fregolino *et al.*, 2011). To date, two genes have been associated with capsule production, *ptk* and *epsA*, and both are required for serum resistance (Russo *et al.*, 2010).

Nutritional immunity is countered by A. baumannii metal acquisition systems

An archetypal example of the host-microbe interface during infection is the struggle for essential nutrient metals. The sequestration of these vital nutrients from invading pathogens is a principal component of host defense against all microbial invaders. Bacteria can tolerate the nutrient-limiting environment by altering metabolic pathways; however, they also directly counter this affront by utilizing diverse nutrient and metal acquisition systems. Bacterial acquisition of non-iron metals is a burgeoning field, but for *A. baumannii,* the little that is known about metal acquisition focuses on iron. In iron-limiting conditions, the canonical iron-sensing repressor Fur releases transcription of iron uptake genes in *A. baumannii* (Daniel *et al.*, 1999). Iron-limiting conditions lead to expression changes not only for iron acquisition genes, but also for genes involved in various processes such as respiration and motility (Eijkelkamp *et al.*, 2011a; Nwugo *et al.*, 2011). The best characterized iron scavenging molecule in *A. baumannii* is the siderophore acinetobactin, which steals iron from transferrin and lactoferrin and is essential for replication in the host (Yamamoto *et al.*, 1994; Gaddy *et al.*, 2012). The systems involved in acinetobactin synthesis, secretion, and import have all been described (Dorsey *et al.*, 2004; Mihara *et al.*, 2004; Zimbler *et al.*, 2009). In addition to siderophore-mediated iron acquisition, putative heme-uptake and ferrous iron uptake systems have been identified in *A. baumannii* (Zimbler *et al.*, 2009; Antunes *et al.*, 2011). Interestingly, distinct clinical strains of *A. baumannii* vary in their expression of many iron acquisition systems including the genes involved in acinetobactin production (Echenique *et al.*, 1992; Actis *et al.*, 1993; Yamamoto *et al.*, 1994). While securing iron via systems described above is critical for *A. baumannii* virulence, *A. baumannii* likely exploits additional strategies to acquire non-iron metals and further circumvent nutritional immunity.

OmpA contributes to multiple aspects of pathogenesis

OmpA or Omp38 is a trimeric outer membrane porin involved in solute transport; however, it is also associated with several aspects of *A. baumannii* virulence. OmpA can be secreted via outer membrane vesicles (OMVs) and contributes to the biogenesis of such vesicles (Jin *et al.*, 2011; Moon *et al.*, 2012). Exposure to these OMVs or purified OmpA induces host cell death by apoptosis. OmpA-mediated apoptosis is attributed to its localization and presumed activity in the mitochondria or DNase activity in the nucleus (Choi *et al.*, 2005; Choi *et al.*, 2008a; Choi *et al.*, 2008b; Lee *et al.*, 2010), although no surface receptors or host protein targets in the nucleus or mitochondria have been identified. Finally, there is evidence that other bacterial proteins may independently contribute to induction of apoptosis in host cells (Gaddy *et al.*, 2009).

OmpA is also immunomodulatory. Although no changes in pro-inflammatory cytokine or chemokine secretion by OmpA-treated cells have been observed, human laryngeal cells up-regulate nitric oxide synthase (iNOS) as well as TLR2 in response to OmpA (Kim *et al.*, 2008). The cytotoxic effects of oxidative stress also provide another connection of OmpA to cell death. At sublethal concentrations, OmpA activates dendritic cells through TLR2 and both MAPK and NF- B pathways, which results in stimulation of $CD4^+$ T cells towards a Th1 response (Lee *et al.*, 2007). In addition to its role in cell death and immune stimulation, OmpA is required for eukaryotic cell adherence and invasion, and partially contributes to biofilm formation and serum resistance (Choi *et al.*, 2008c; Gaddy *et al.*, 2009; Kim *et al.*, 2009). Figure 1 summarizes the numerous roles attributed to OmpA as an *A. baumannii* virulence factor. While the possibility exists that OmpA is multifunctional, the number of distinct processes in which OmpA is involved suggests that some of these ascribed effects are indirect.

Multiple virulence determinants permit A. baumannii to flourish in the host

In addition to numerous proteins involved in antimicrobial resistance, other *A. baumannii* proteins have been described that enable *A. baumannii* to persist in the host. Sequencing and bioinformatic analyses of the genomes of several *A. baumannii* strains have led to the identification of other putative virulence factors (Fournier *et al.*, 2006; Smith *et al.*, 2007; Adams *et al.*, 2008; Iacono *et al.*, 2008; Vallenet *et al.*, 2008). In several cases, functional expression of these potential virulence determinants has not been confirmed or expression is not universal among strains and clinical isolates. This latter aspect underscores the genomic plasticity observed among *Acinetobacter* species and strains of *A. baumannii*. Comparison of *A. baumannii* ATCC 17978 and *A. baylyi* ADP1 has revealed at least 28 putative pathogenicity islands that encode antibiotic resistance determinants and potential virulence genes (Smith *et al.*, 2007). The field has since acquired the genome sequences for numerous strains of *A. baumannii.* Comparative studies of these genomes have identified frequent genetic differences among strains that highlight the pronounced capacity for horizontal gene acquisition and genome rearrangement that likely account for the variation in pathogenicity (Imperi *et al.*, 2011). In this regard, genomic analyses suggest that *A. baumannii* encodes groups of proteins with homology to type IV and type VI secretion systems, which are often associated with virulence in other bacterial pathogens (Smith *et al.*, 2007; Henry *et al.*, 2011). Furthermore, in response to DNA damage and oxidative stress experienced by *A. baumannii* in the host, the DNA repair protein RecA is required for bacterial survival (Aranda *et al.*, 2011). Finally, a phospholipase D protein, a phospholipase C protein, and a sensor kinase GacS are necessary for full virulence in animal models (Peleg *et al.*, 2008b; Camarena *et al.*, 2010; Jacobs *et al.*, 2010).

An important aspect of pathogenesis is transmission to a host, which is enhanced through bacterial biofilm formation on abiotic surfaces such as catheters and ventilators. Several factors involved in the different phases of biofilm formation have been identified in *A. baumannii*. First, Type IV pili have been implicated in motility, which would allow for spread to new surfaces (Eijkelkamp *et al.*, 2011b). The CsuA/BABCDE chaperone-usher pili assembly system is required for adherence to abiotic surfaces, though not for adherence to eukaryotic cells (Tomaras *et al.*, 2003; de Breij *et al.*, 2009). Following adherence, the production of the biofilm extracellular matrix is dependent on synthesis of the polysaccharide poly- -(1,6)-*N*-acetylglucosamine (PNAG) (Choi *et al.*, 2009). The outer membrane protein Bap is necessary for biofilm maturation and also for adherence to eukaryotic cells (Loehfelm *et al.*, 2008; Brossard *et al.*, 2011). Regulation of biofilm formation and motility involves the regulatory elements BfmSR and an *N*-acyl-homo-serine lactone (AHL) signaling molecule (Niu *et al.*, 2008; Tomaras *et al.*, 2008; Clemmer *et al.*, 2011; Eijkelkamp *et al.*, 2011b). Although all of these proteins may not be essential within the host, biofilm formation by *A. baumannii* alters expression of proteins involved in numerous activities, including those involved in other aspects of *A. baumannii* virulence (Shin *et al.*, 2009; Cabral *et al.*, 2011; Marti *et al.*, 2011). This latter point emphasizes the importance of motility and biofilm formation to virulence.

Conclusions and future directions

The increasing threat of *A. baumannii* infections in hospitals combined with the decreasing capacity to effectively treat antibiotic resistant strains has fueled *A. baumannii* research. Significant strides have been made towards understanding its antibiotic resistance and its ability to survive in the hospital environment. On the other hand, there is a paucity of information about host-*Acinetobacter* interactions and the mechanisms by which they impact pathogenesis. Research in this area should be facilitated through the employment of the established pneumonia and sepsis models as well as the recently developed rat soft tissue infection model (Russo *et al.*, 2008). Several other models that have been useful in the identification of *A. baumannii* virulence determinants include the amoebae *Dictyostelium discoideum* (Smith *et al.*, 2007), the nematode *Caenorhabditis elegans* (Smith *et al.*, 2004), and larvae of the insect *Galleria mellonella* (Peleg *et al.*, 2009). These models have been useful to investigate the general host response; however, studies aiming to delineate innate immune signaling pathways have been limited. Furthermore, the environment of the immune-compromised host and the contribution of the anti-inflammatory response to controlling damaging inflammation have important implications to pathogenesis that we are only beginning to appreciate. Immunomodulatory therapeutics in patients with compromised immune status may be a beneficial strategy for treatment of *Acinetobacter* infections. However, the development of such therapies is dependent on a more complete understanding of the host immune response to *A. baumannii*. Antigen presentation to the host immune system depends on the intracellular or extracellular localization of *A. baumannii* within the infected tissue. Beyond the ability to adhere to and enter cells, virtually nothing is known about the intracellular lifecycle and trafficking of *A. baumannii* or the bacterial and host factors mediating these processes. For many bacterial pathogens, the virulence factor repertoire includes proteins that directly modulate the host immune response. Other than OmpA, bacterial proteins that directly manipulate the host have yet to be identified. Nevertheless, the heterogeneity of the survival strategies and virulence factors exhibited by *A. baumannii* strains highlights the adaptability of the organism to a variety of host assaults. Figure 2 illustrates both the host and bacterial defense tactics employed at the frontline of the host-pathogen battlefield existing during an *A. baumannii* infection. The recent development of bacterial genetic tools and animal infection models should help address the deficiencies in our understanding of *Acinetobacter* virulence. Knowledge gained from such

research will facilitate the identification of drug targets and guide the design of effective therapeutics to target this emerging threat.

Acknowledgments

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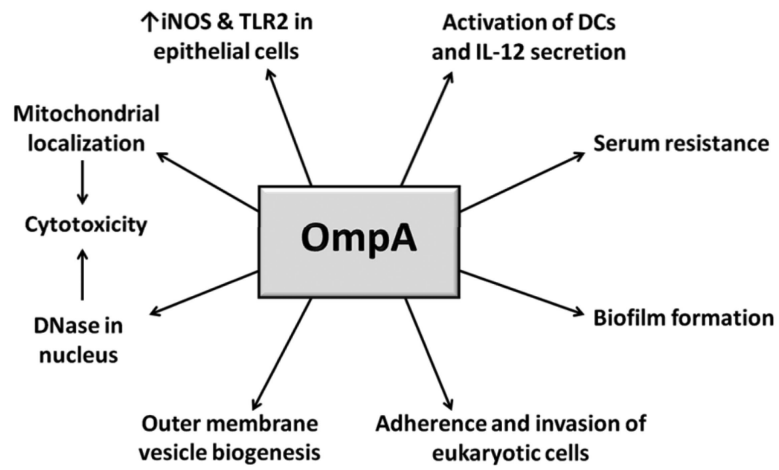
Figure 1.
Outline of the multiple contributions of *A. baumannii* OmpA to pathogenesis.
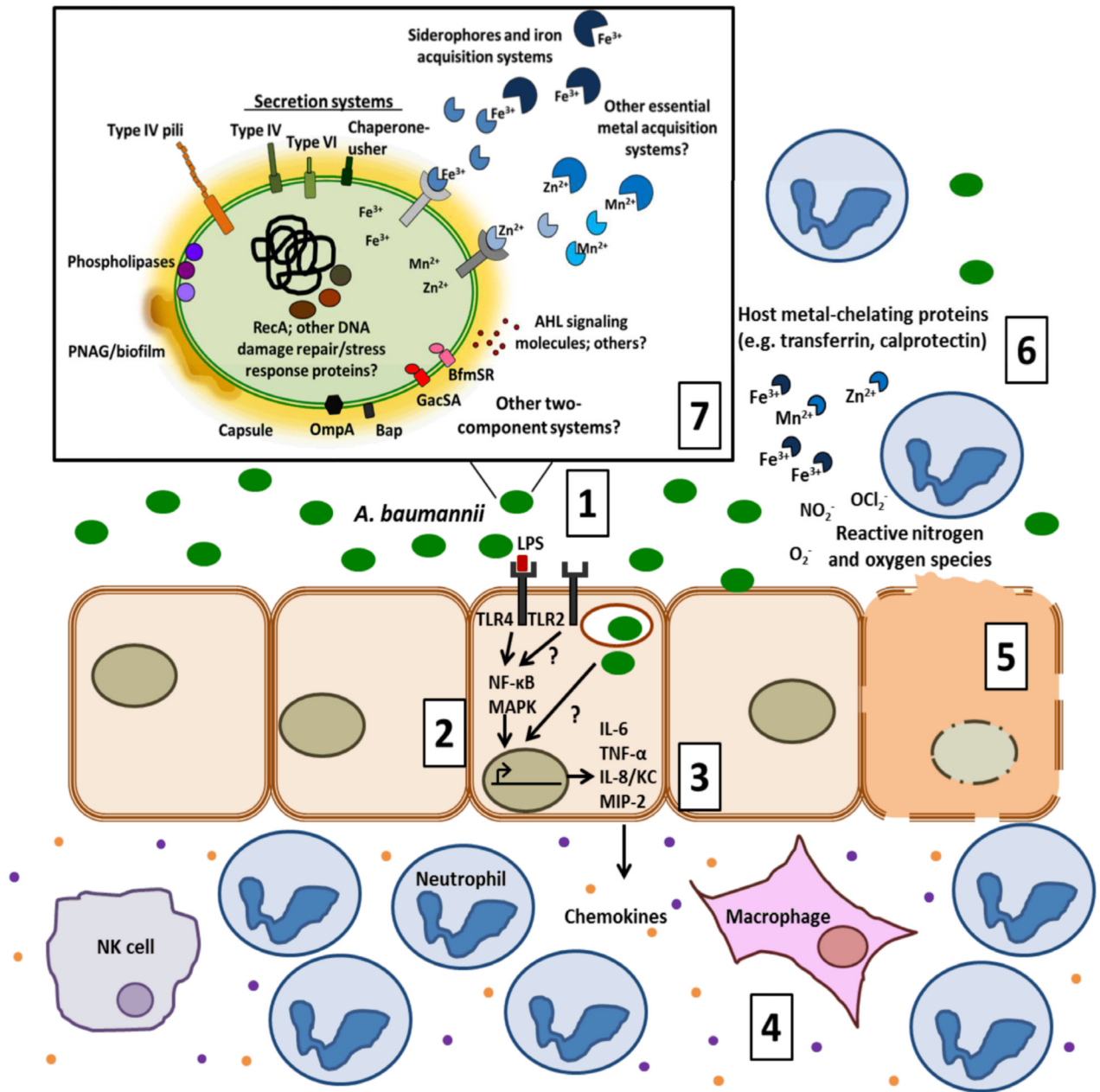
Figure 2. Summary of the dynamic interplay between *A. baumannii* and the host
(1) *A. baumannii* can adhere and invade host cells, leading to stimulation of the pro-inflammatory immune response. (2) The inflammatory response is initiated by TLR4 recognition of LPS which then activates MAPK and NF- B pathways. TLR2 is also reported to detect *A. baumannii*. (3) Activation of these receptor proteins leads to subsequent transcription and secretion of pro-inflammatory mediators such as cytokines IL-6 and TNF- and chemokines KC/IL-8 and MIP-2. (4) These chemokines recruit granulocytes and lymphocytes that are required for controlling infection. (5) Following *A. baumannii* infection host cells also undergo apoptosis. (6) Other host defenses include nutritional immunity, ROS/RNS production, and antimicrobial peptides. (7) In response to the host environment, *A. baumannii* expresses several virulence factors implicated in pathogenesis, which are displayed in the inset of the figure. The illustration depicts those

proteins and molecules that are functionally characterized and those that are predicted to be expressed. The question marks designate areas in which there are significant gaps in our knowledge.